



FINAL REPORT

Lanthanide-Containing Cyclophanes for the Detection of Explosives and Propellants

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Acronyms and Abbreviations

acac	acetylacetone
BHEEN	1,5-bis[2(2-hydroxyethoxy)ethoxy]naphthalene
DMF	<i>N,N</i> -dimethylformamide
DNA	deoxyribonucleic acid
DNT	dinitrotoluene
DoD	Department of Defense
g	gram
h	hour
hfac	hexafluoroacetylacetone
HMX	cyclotetramethylenetrinitramine
λ	wave length
mL	milliliter
M	molarity
mM	millimolarity
mmol	millimole
ms	millisecond
μ s	microsecond
nm	nanometer
NMR	nuclear magnetic resonance
OAc	acetate
RDX	cyclotrimethylenetrinitramine
TNT	trinitrotoluene
UV-vis	ultraviolet-visible

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Executive Summary

Testing ranges are subject to increased exposure from propellants and explosives as these chemicals leach from ordinance shells. Consequently, the neighboring ground water is at risk of contamination from these pollutants. In order to identify areas that require clean up or are at risk of high levels of these contaminants, a chemical assay is first needed to provide a rapid test for the specific analytes of concern. A chemical assay can then be adapted to other forms of deployment such as dipstick or spray-on techniques for screening localized or large areas, respectively. An efficient assay provides a rapid diagnosis of the sampled soil or ground water with a minimum chance of false positives.

In order to transduce the presence of a propellant or explosive into a readable output, a receptor must selectively interact with the particular chemical of interest. Subsequently, the binding event triggers a spectroscopic or electrical response within the receptor, which provides the output signal indicating positive identification. The selectivity of the receptor for the specific propellants and explosive determines the rate of false positives in the chemical assay. However, this selectivity must be balanced with high sensitivity to avoid false negatives. For example, a highly selective receptor may require high concentrations of TNT in order to indicate a positive response to the explosive and low levels of TNT may be undetectable. Similarly, a highly sensitive sensor may indicate a false positive response for innocuous chemicals found in the environment.

A functionalized cyclophane receptor was synthesized to selectively bind aromatic explosives such as TNT. The organic cyclophane is functionalized with ligating capabilities along its periphery, which allow for the binding of lanthanide chromophores. The fluorescence properties of lanthanides are significantly altered upon binding of the aromatic analytes into the cavity of the cyclophane. The excited-state lifetime decreases by approximately 30 % when the cyclophane binds electron-deficient aromatics such as dinitrotoluene and dinitrobenzene, which are decomposition products of TNT. By contrast, electron-rich aromatics such as toluene, biphenyl, and naphthalene, which may act as interferents, induce a small increase in the lifetime of the lanthanide. Thus, the cyclophane receptors provide selective detection of nitroaromatics. Furthermore, the sensitivity of the lanthanide-cyclophane complex to the aromatics is better than 10 ppm. *As a chemical assay, the cyclophanes can distinguish the nitroaromatics indicative of explosives from aromatic hydrocarbons with high sensitivity.*

1. OBJECTIVE

The object of this proposal is to demonstrate a fieldable, screening-level chemical assay for nitroaromatic explosives and their decomposition products (2,4,6-trinitrotoluene (TNT), 2,4-dinitrotoluene (DNT)). The optical response of lanthanide-modified cyclophanes will transduce the presence of the analytes into a readable output. The macrocyclic structure of the cyclophanes provides the element of selectivity to the chemical assay. These chemical technologies will form the basis of an optical “dipstick” sensor that can be used to rapidly test for explosive and propellant contamination in soil, ground water, and surface water. Alternatively, the new assay technology can be adopted for “spray-on” usage for more standoff capabilities.

2. BACKGROUND

Military training exercises utilize a variety of explosives and propellants such as RDX, TNT, HMX, and perchlorate that can lead to groundwater contamination on DoD ranges. Perchlorate has been detected in groundwater near Mather and Edwards Air Force Bases and in surface waters including the Colorado River.¹ Many of these contaminants are known toxics while others have unknown risks. Millions of acres of training areas need to be tested for high levels of energetic pollutants. Rapid, screening-level field tests for these compounds are currently unavailable. In order to accurately detect the presence of explosives or propellants in soil or ground water, samples must be taken back to the laboratory for expensive and time consuming analysis. To identify areas that require decontamination, a field test is needed which rapidly detects and quantifies the presence of explosive materials.

Once developed, a chemical assay can lead to more diverse testing and detection methods such as dipstick or spray on techniques. These additional methods will allow the chemical assay technology to be adopted for screening larger areas with standoff capabilities. Before they can be implemented, the basic chemical assay must first be developed. A chemical assay contains two important elements, a receptor that provides selectivity towards the analyte of interest and a transduction mechanism that indicates a positive response, the activation of the receptor.² The receptor can be a chemical reaction that selectively reacts with a specific functional group or a biomolecule that forms a supramolecular complex with the analyte. Upon binding or reaction of the receptor, the transduction element produces a readable output. The output can be in the form of an electrical response as in lab-on-a-chip technologies or, more conveniently, in the form of an optical read out.

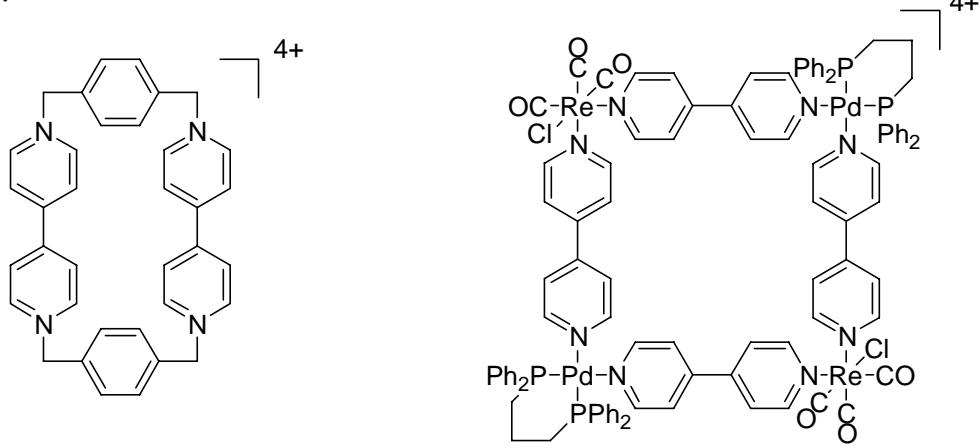
Fluorescent indicators provide highly sensitive chemical assays that can detect analytes with parts-per-billion and parts-per-trillion sensitivities.³ With such high sensitivities, the probability for a false positive is increased. Consequently, the applicability of most fluorescent assays is limited by their selectivity. One strategy for overcoming this limitation is the use of separation methods that remove impurities from the analyte that might give rise to false positives.⁴ This approach requires added levels of engineering, cost, and time. Moreover, isolation of the analyte is not an option with standoff detection. Rapid screening for environmental contamination requires a more selective receptor.

Supramolecular interactions that utilize a lock-and-key mechanism are rapidly becoming the preferred method for detecting and identifying chemical analytes.³ In these devices, a receptor is engineered to form a guest-host complex with a targeted molecule. Binding of the analyte (guest) induces changes in the optical or electrochemical properties of the host. These observable phenomena transduce the presence of the analyte into a readable output. Biochemical assays utilize supramolecular binding to detect specific proteins, antibodies, antigens, or DNA sequences with fluorescence, radio, and enzyme immunoassays⁴. In these examples, naturally occurring host-guest complexes are

identified and used to develop the assay. The high selectivity of the biomolecular recognition process enables single analytes to be discerned from the background of a complex biological fluid.

Artificial supramolecular systems are currently being developed to identify chemical analytes with the high selectivity of naturally occurring bio-recognition systems⁵. Many complexing agents have been synthesized to bind specific molecules while others are engineered to interact with entire classes of compounds. Unfortunately, the detection of the guest-host complex has predominately relied on costly and time-consuming NMR measurements. A timely electric or optical response is needed to quickly read the output of the sensor for practical applications. Ideally, an assay will provide a rapid, inexpensive, and convenient test for the supramolecular complex.

Both organic and inorganic structures have been engineered to contain cavities and pores large enough to accommodate smaller molecules.^{3,6} Cyclic compounds such as **1** and **2** are known to bind small ions and aromatic organics.^{7,8} Analytes bind to the center of the cavity through nonspecific interactions such as hydrophobic effects or coulombic attraction. The binding and proximity of the guest causes a perturbation in the electronic structure of the host, which can result in measurable changes in the electronic and optical properties of the complex. For example, complexation of aromatics by **1** results in a dramatic change in the chemical shift (> 2.5 ppm) of the protons in the guest molecule. Similarly, the binding of perchlorate to **2** results in an enhancement of the emission intensity.



1

2

While many organic cyclophanes are also easily prepared, few, such as **1** above, contain redox centers that are responsive to the presence of analytes within the molecular cavity. Unfortunately, most organic reagents lack a reversible electrochemistry or a convenient spectroscopic handle for probing guest-host interactions. For sensor applications, the association of the analyte molecules to the cyclophane must be coupled to a transduction mechanism that converts the binding event into a readable output. One solution to this problem is to incorporate metal centers into the organic framework of the macro cycle.

The majority of metallacyclic squares such as **2** are prepared using rational synthetic protocols that are unique to each series of compounds. One advantage of the metal-based systems is the presence of the transition metal centers, which often possess electrochemical and photophysical properties that are highly sensitive to slight changes in chemical environment. These attributes are ideal for developing sensor applications since they provide a built-in transduction mechanism for converting the presence of the analyte into a readable output. Unfortunately, the heavy transition metals are extremely toxic and expensive. These qualities will limit the application of these devices as field probes for chemical detection.

By contrast, no metallacycles have been reported for the f-block metals. The fluorescent properties of the lanthanide ions (Tb^{3+} , Dy^{3+} , Eu^{3+} , and Sm^{3+} for example) are highly sensitive to perturbations in the electrostatic environment around the metal. The lanthanide ions are inherently weak absorbers of light due to their formally-forbidden $f-f$ electronic transitions. However, when chemically complexed with suitable organic chromophores, strong absorption of incident light followed by efficient intra-molecular energy transfer results in moderately intense emission of narrow-band visible and near-infrared radiation from these ions. The efficiency of intra-molecular energy transfer depends to a large extent on the molecular triplet state⁹ and its energetic proximity to the lanthanide excited state, the physical distance between the chromophore and the lanthanide ion, and the existence of competing, non-radiative pathways such as vibronic quenching. Accordingly, the photophysical properties of a given lanthanide complex can be readily influenced by a variety of factors including chemical binding, solvent effects, and changes in molecular conformation.

As fluorescent probes, lanthanide complexes offer several attractive advantages. Most lanthanide complexes feature large Stokes shifts, i.e., substantial wavelength separation between excitation and emission. Consequently, it is possible to detect weak emissions in the presence of exciting radiation using photonics devices of only moderate spectral resolution. The specific lanthanide ions listed above feature long-lived fluorescence that in conjunction with gated detection enables a high degree of discrimination against short-lived fluorescent backgrounds and rejection of scattered light, Table 1. The fluorescent peak profiles of lanthanide ions are relatively sharp with half-widths in the range of 10-20 nm (Figure 1).

Table 1. Fluorescent Lifetimes of Lanthanide Ions

Lanthanide	Complex Excitation (nm)	Ln^{3+} Emission (nm)	Fluorescence Lifetime τ (μ s)*
Terbium (Tb)	320	545	1050
Dysprosium (Dy)	320	572	16
Europium (Eu)	350	615	730
Samarium (Sm)	350	642	50

*The fluorescent lifetime of a particular lanthanide is ligand dependent

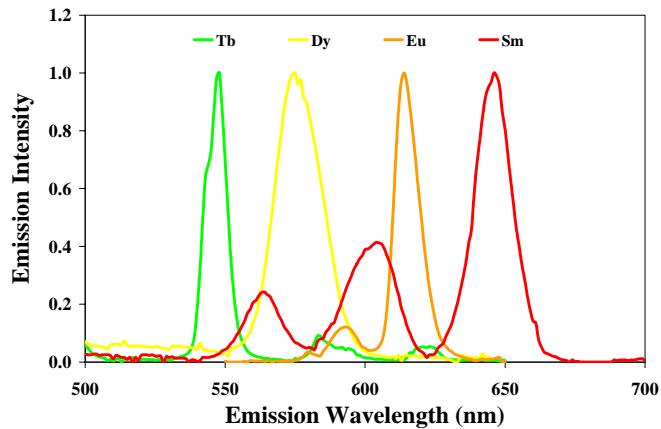


Figure 1. Narrow-band Visible Emission from Lanthanide Ions

As a result of the favorable properties listed above, lanthanide complexes have seen widespread use as fluorescent labels for biological immunoassay applications, and are ideally suited for a variety of environmental sensing applications as well. One of the appealing features of many lanthanide complexes is the ability to attach these species to other molecules.¹⁰ Perturbations to the host molecule will affect to some degree the fluorescent characteristics of the attached lanthanide complex. This effect may be manifested in one of three ways. The emission wavelength of the lanthanide ion is generally intrinsic, and consequently, any shifts in emission wavelength associated with the molecular environment are likely to be subtle. Much more pronounced are changes in the emission intensity and the fluorescent lifetime of the lanthanide ion due to alteration of the energy transfer process and quenching effects, respectively. Small changes in fluorescent lifetime are more easily quantifiable since they are completely independent of the intensity of the exciting or emitted radiation. Measuring changes in the intensity of the emitted radiation, although feasible, require an optical reference to account for changes in the exciting source intensity.

3. MATERIALS AND METHODS

3.1 Materials

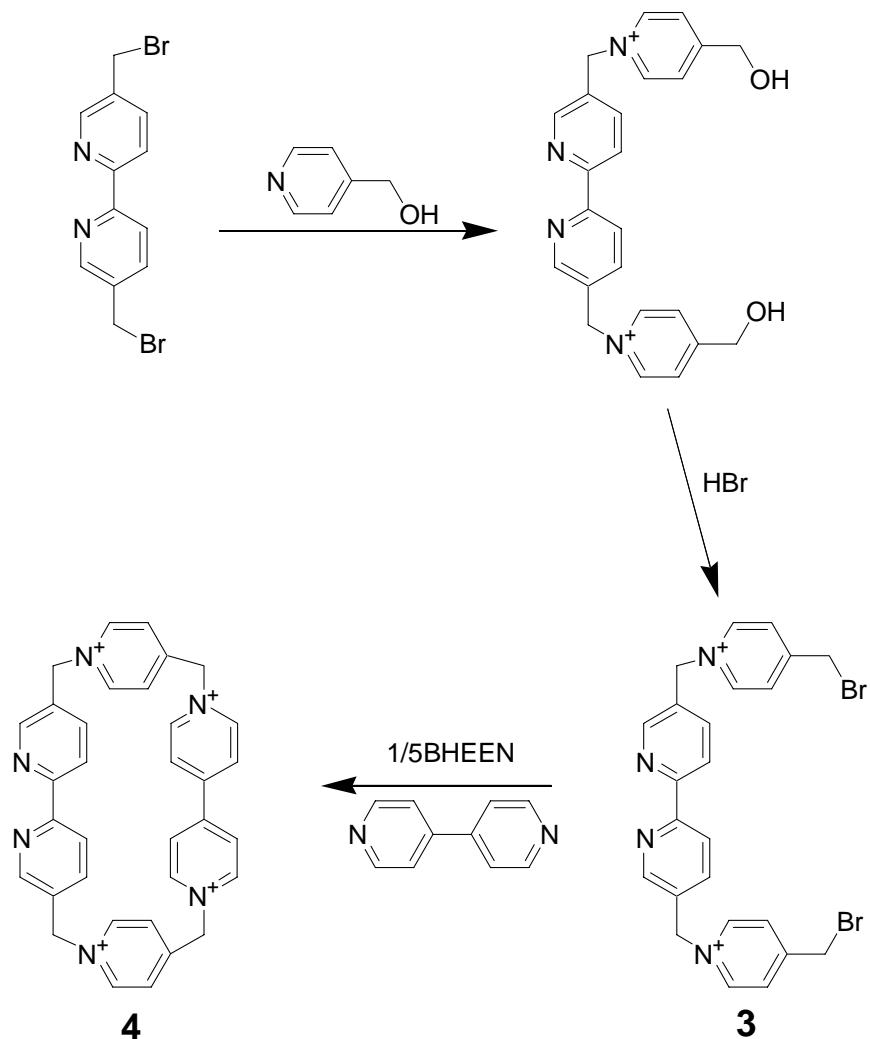
Unless otherwise noted, all chemicals were used as received from Aldrich. 5,5'-Bis(bromomethyl)-2,2'-bipyridine and 1,5-bis[2(2-hydroxyethoxy)ethoxy]naphthalene (1/5BHEEN) were prepared using literature methods.^{13,14}

3.2 Instrumentation

Fluorescence measurements were obtained with a FluoroMax-P spectrofluorometer and UV-Vis spectra were acquired using a Cary 5E UV-Vis-NIR Spectrophotometer.

3.3 Cyclophane Synthesis

The functionalized cyclophane was synthesized according to Scheme 1:



3.3.1 Preparation of Cyclophane 1

Step 1

5,5'-Bis(bromomethyl)-2,2'-bipyridine (0.50 g, 1.46 mmol) and 4-pyridinemethanol (TCI America, 0.34 g, 3.12 mmol) were dissolved in 40 mL acetonitrile. The reaction was heated at reflux, under nitrogen, for 46 h. The cooled suspension was filtered through a medium-porosity fritted glass funnel; the filtered crude precipitate was the desired product, sufficiently pure for the next step, according to NMR analysis. (The acetonitrile filtrate contained essentially only excess 4-pyridinemethanol.)

Step 2

The 5,5'-bis[(4-hydroxymethyl)pyridinylmethyl]-2,2'-bipyridine product (0.20 g, 0.26 mmol) in 3.5 mL of 48% aq. HBr was heated at reflux, under nitrogen, for 4 h one day and 1 h on the next day. Solid that collected in the condenser during reflux was repeatedly pipetted back into the reaction flask using the reaction solution. The reaction solution was then concentrated to dryness under high vacuum.

The crude product was recrystallized by dissolving in ~3 mL water followed by addition of 20 mL acetonitrile. A reddish oily layer plus light pink solid resulted. The supernatant was decanted into a medium-porosity fritted glass funnel, giving a pink solid (first crop) and clear filtrate. The oily layer was redissolved in ~1 mL water and acetonitrile was added until a flocculent pink solid appeared. The suspension was boiled and then cooled. The dark pink solid (second crop) was filtered with a medium-porosity fritted glass funnel, which gave a cloudy filtrate. The cloudy filtrate was filtered with a fine-porosity fritted glass funnel, which collected a light pink solid (third crop), leaving very slightly cloudy filtrate. The filtrate from the first filtration of the first crop produced a transient white precipitate during concentration by rotary evaporation. Upon drying, the white precipitate turned into a pale yellow oil. All collected products (three crops plus oil) were vacuum-dried over phosphorus pentoxide over a weekend. NMR analysis in D₂O of all portions revealed the desired brominated product with only slight impurities. (¹H NMR of the product is very pH-dependent, including the pattern of the aromatic region and the chemical shifts of the methylene protons. In a certain pH range, the bromomethyl CH₂ protons exactly overlap residual protons of HOD in D₂O, but they can be shifted by addition of DBr in D₂O.)

The crude product crops were collected together and filtered through their respective frits with dilute (~5%) aqueous HBr. (NOTE: On scale-up, if a white precipitate or oil was produced upon concentration of a filtrate, the white precipitate or oil should not be added in with pink precipitates at this stage.) The aqueous solution was “neutralized” to pH 6 with aqueous 1.0 M tetrabutylammonium hydroxide, producing a pink precipitate and a pink solution. (NOTE: On scale-up, the excess aq. HBr should be removed under vacuum, and the solute should be redissolved in water prior to neutralization, thereby avoiding excessive base.) The aqueous precipitate was filtered with a medium-porosity fritted glass funnel and vacuum-dried over phosphorus pentoxide. This solid weighed only 10 mg and proved not to be useful due to its low solubility in acetonitrile. The pink filtrate was evaporated to dryness, leaving an off-white powder, which mostly dissolved

in ~100 mL acetonitrile. Pink solid that was suspended in the acetonitrile was filtered off (medium-porosity frit) and vacuum-dried over phosphorus pentoxide, and the filtrate was colorless. The collected product was stirred in ~50 mL water over a weekend, and then a small amount of insoluble light-orange solid was filtered off. The aqueous filtrate was concentrated to dryness under vacuum. Concentration produced a small white clump that was segregated from the majority of solute, and the clump was physically removed. (NMR analysis revealed it to be a reaction byproduct.) The remaining solid analyzed correctly for the desired product **3** by elemental analysis (C, H, N, Br) and NMR.

Step 3

The final reaction step to close the cyclophane follows a procedure similar to that of Ashton et al (method C)¹⁵ using the ¾ cyclophane with 4,4'-bipyridine and 1/5BHEEN as the template molecule along with a catalytic amount of NaI. A 100ml round bottom flask fitted with magnetic stir bar and a ground glass stopper was charged with **3** (0.784g, 0.96mmol), 4,4''-bipyridine (0.150g, 0.96mmol), 1/5BHEEN (0.969g, 2.88mmol) and 10mg NaI (0.07mmol). Dry *N,N'*-dimethylformamide (DMF) (40mL) was added and the solution was allowed to stir for 11 days at ambient temperature. The progress of the reaction was monitored by ¹H NMR. (An aliquot was removed from the mixture, the DMF removed in vacuuo, and the sample dissolved in D₃-acetonitrile with an excess of KPF₆. The reaction progress was observed by the disappearance of the -CH₂Br peaks at about 4.6 ppm.) After stirring for 11 days the DMF was removed in vacuuo and the compound precipitated from water by adding excess KPF₆. The compound was then extracted into dry acetonitrile, filtered and dried to give 0.682 g of the PF₆⁻ salt of the cyclophane **4** (60 % yield, C₃₄H₃₀H₆P₄F₂₄). The compound was a colorless solid that generally produced some deep blue color on dissolution which slowly turned to a brown color on sitting in solution exposed to the air and produced a tan solid on drying.

3.3.2 Lifetime Studies

Solutions containing TbCl₃, **4**, and an aromatic analyte were prepared with methanol. Equal concentrations of Tb³⁺ and **4** were maintained between 0.01 and 0.3 mM. Analyte concentrations varied from 9-90 ppm. Solutions were excited at wavelengths near the absorption maximum (250-300 nm) as determined by UV-Vis spectroscopy. Emission spectra were recorded with the detector aligned 90° to the incident radiation. The excited state lifetimes of the Tb³⁺ were monitored as a function of the analyte solutions.

4. RESULTS AND ACCOMPLISHMENTS

4.1 Cyclophane Synthesis and Metalation

The cyclophane **4** was synthesized using modified procedures for the preparation of other templated organic cyclophanes. Reaction Scheme 1 provides useful quantities of material in relatively high yields with only small amounts (< 5%) of impurities that were identified predominately as unclosed cyclophane species via H-NMR spectroscopy. Fortunately, these impurities do not interfere with the spectroscopy of the pure compound nor interfere with its detection capabilities *infra* *vida*.

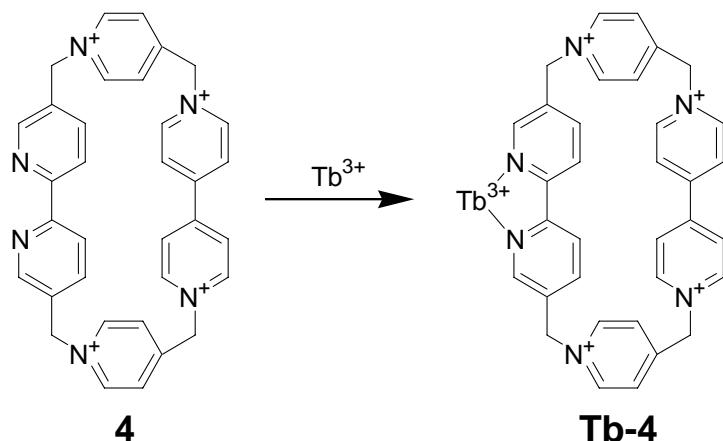
The presence of the non-alkylated bipyridine group in the cyclophane precursors was of concern since these groups can react with the alkyl bromide substituents (Step 1 and 3). The product of these side reactions are misdirected alkylation products. Moreover, these unwanted alkylation products would likely be insoluble polymeric species and decrease the overall yield of the synthetic process. Fortunately, the steric hinderence of the pyridyl groups significantly retards the rate of these unwanted reactions to the point where they no longer inhibit the successful synthesis of **3**. Generation of cyclophane **4** with these seemingly incompatible functional groups greatly simplifies the reaction scheme by limiting the need for protecting groups.

During the synthesis of **4**, we noticed that solutions of the alkylated precursors take on various colors depending on the solvent and counter ion. These solvochromic and ionochromic properties are indicative of a charge-transfer process.¹⁶ Presumably, charge transfer takes place between the unalkylated dipyridyl group or counter anions and the alkylated pyridiniums. Similar chromic effects are known for simple dialkylated dipyridinium salts.¹⁷ The presence of these low-energy absorptions provide a potential mechanism for quenching the emission of any pendent lanthanides in a chemical assay.

Combining **4** and TbX_3 ($\text{X} = \text{Cl}$, acetate (OAc), acetylacetone (acac), hexafluoroacetylacetone (hfac)) in methanol results in significant color changes depending on the nature of X . Solutions of the PF_6^- salt of **4** are faintly colored yellow depending on the solvent. However, addition of TbOAc_3 and Tb(acac)_3 result in a deep blue color, indicative of the reduced radical cation form of the dipyridinium salts.¹⁷ In these cases the anions OAc^- and acac^- reduce the dialkylated dipyridinium dication. Addition of oxidizing agents (hydrogen peroxide) removes the blue color. By contrast, addition of the chloride and hexafluoroacetylacetone salts of terbium(III) results in a faint pink solution. In these cases, the oxidation potentials of the Cl^- and hfac^- anions are too high to reduce the dipyridinium dication. Rather, the color change from yellow to pink is likely due to the coordination of the bipyridine ligand of **4** to the metal center. Since, Tb(hfac)_3 and TbCl_3 do not partake in redox reactions with **4**, these complexes were used in metalation studies of **4**. NMR spectra on quantitative mixture of **4** and TbCl_3 or Tb(hfac)_3 in methanol, acetonitrile, water, or dimethylsulfoxide reveals that the metal center coordinates to the cyclophane. Due to the paramagnetic nature of terbium, the shift in the NMR spectra is due to a combination of the change in electronic density

on the pyridyl ligand of **4** and the paramagnetic shift of the Tb. However, an excess of **4** produces a second set of peaks in the NMR spectra suggesting that the Tb³⁺ centers coordinate to **4**, Scheme 2. Unfortunately, the paramagnetic shift precludes a quantitative determination of the binding ratio of Tb³⁺ and **4**. Presumably, solvent molecules and counter anions fill the coordination sphere of the metal. Since these ligands are not expected to influence the photophysical properties of the metal, no effort was made to identify the full coordination sphere of Tb-**4**

Scheme 2



These results indicate that the Tb³⁺ is coordinated to the cyclophane. As a chemical assay, the cyclophane will serve as the receptor and changes in the photophysical properties of the Tb³⁺ will act as the transduction mechanism. *By coordinating the terbium to the cyclophane we have successfully attached the receptor to the transduction mechanism.* Subsequent work focuses on the photophysical properties of the terbium-cyclophane Tb-**4** complex.

4.2 Photophysical Properties of Tb-4

Addition of Tb³⁺ to a solution of **4** results in a color change due to a perturbation of the charge-transfer properties of **4**, Figure 2. Coordination of the metal center to the dipyradyl unit of **4** alters the donor properties of the coordinating ligand and, hence, changes the charge-transfer energy. The intense absorption at 280 nm for **4** is associated with a pi-pi* transition of the two dipyradyl units. The high energy band is essential for successful energy transfer to the terbium center. Since the f-f* transition of terbium is formally forbidden, the pi-pi* transition of the cyclophane acts as an antenna. The excited cyclophane efficiently transfers energy to the terbium generating the excited lanthanide. The energy transfer is only possible if the pi* energetic state is sufficiently high enough in energy. Importantly, coordination of the metal center does not significantly shift the energy of the pi* state. Absorptions at lower energies (500-650 nm)

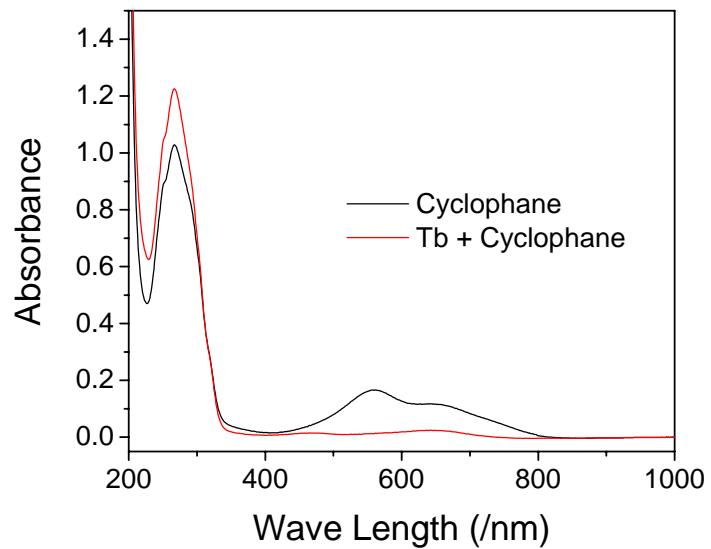


Figure 2. UV-Vis spectra of **4** (black) and **Tb-4** (red) in methanol.

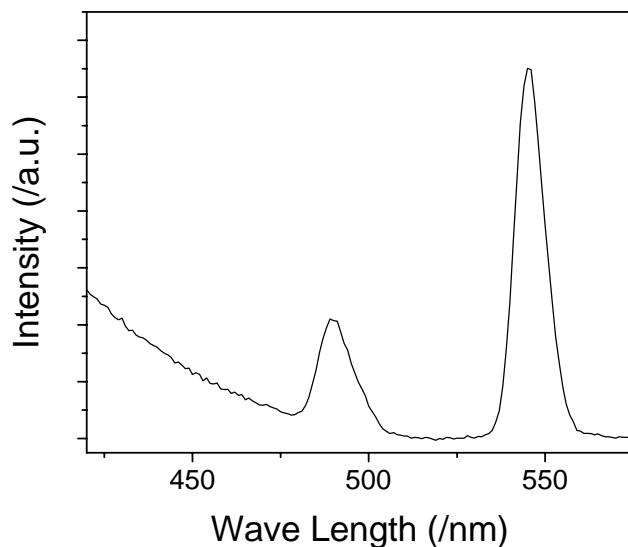


Figure 3. Emission spectrum of **Tb-4** (0.1 mM) in methanol. Excitation energy is 280 nm

are the charge-transfer band. These energies are highly dependent on the nature of the counter anion and solvent and are significantly diminished upon coordination of the terbium. Moreover, these charge-transfer energies and their high sensitivity to chemical analytes can serve as quenchers to the emission of the excited terbium centers.

The efficient energy transfer of the excited pi-pi* transition to the terbium centers is evident by the emission spectrum of **Tb-4**, Figure 3. The emission spectrum with λ_{max} of 545 nm is indicative of fluorescent radiation from Tb^{3+} (compare Figures 1 and 3). The high energy emission ($\lambda < 475$ nm) is the short lifetime decay associated with the solvent and cyclophane. The decay rate for these fast quenching processes is less than 1 μs . By comparison, the decay rate of the 545 nm emission is 1000 times longer on the order of 1 ms. This intense emission at 545 nm is the spectroscopic output of the chemical assay. Changes in the intensity or lifetime of the 545 nm emission are expected to occur as analytes bind to the cyclophane receptor. Since changes in emission intensity vary with the intensity and energy of the incident radiation and require an optical reference, changes in the lifetime of the excited state are more conveniently compared. Specifically, changes in the excited state lifetimes will be quantified and monitored for as a function of the analyte molecules. The high intensity of this emission enables minimal amounts of **Tb-4** to be used in the chemical assay and any related technologies derived from the test will similarly require small amounts of **Tb-4**.

Transient measurements of the emission at 545 nm reveal an exponential decay (Figure 4). The transient data can be fit to a single exponential decay to extract the half-life of the terbium excited state. The high quality of the data fit indicates that the quencher of the Tb centers is homogenous within the sample. For example, the emission from terbium centers with two cyclophanes coordinated to the metal center would be quenched significantly faster than **Tb-4** which has only a single cyclophane. Similarly, a mixture of terbiums with one, two, or more cyclophanes would emit 545 nm radiation with three different decay constants. Fitting of the observed data to only one decay constant is consistent with a single uniform arrangement of cyclophanes about the metal centers. Importantly, the good fits also suggest that only a single source is responsible for the emission at 545 nm. Impurities such as unclosed cyclophane portions that can bind to emitting Tb^{3+} centers do not significantly convolute the fluorescent signal of the receptor/transducer of **Tb-4**. Any impurities that also fluoresce at 545 nm on this timeframe can complicate the decay curves and obscure the assay results. In addition, the impurities may quench the decay of **Tb-4** by binding to the cyclophane receptor. However, the measured signal is relatively intense indicating minimal affect of any impurities.

Most important for sensor applications are the emission properties of **Tb-4** in the presence of nitroaromatics and aromatic hydrocarbons. The structure of the cyclophane is designed to loosely bind electron deficient aromatics such as nitroaromatics within the cavity created by the organic ring. The electron-rich aromatic rings of the cyclophane promote the binding for electron-deficient aromatics through quadrupole-quadrupole interactions. Binding of the nitroaromatic is expected to perturb the geometry and electronic structure of the cyclophane which is then reflected as a change of the lifetime

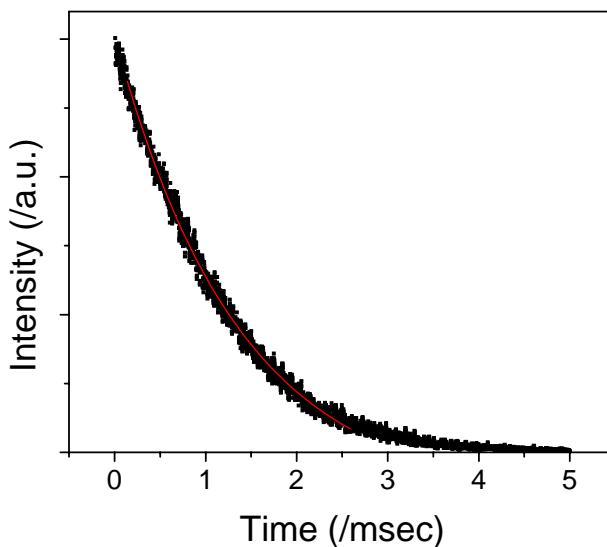


Figure 4. **Transient decay of the 545 nm emission of Tb-4 (0.1 mM) in methanol with 285 nm excitation (The red line is a fit of the data (black dots) to a single exponential decay)**

of the terbium excited state. However, other weak intermolecular interactions such as van der Waals forces can allow for interferents (electron-rich aromatics) to be encapsulated by the cyclophane. Fortunately, the unwanted binding of an electron-rich hydrocarbon does not guarantee a positive response as the perturbation of the cyclophane is unlikely to mimic exactly the electron deficient aromatics.

The emission decay for methanol solutions of Tb-4 in the presence of the nitroaromatics and hydrocarbons are significantly different, Figure 5. The half-life for Tb-4 in methanol is 1.36 ms as determined by fitting the data to an exponential decay. Addition of aromatic hydrocarbons such as toluene, naphthalene, and biphenyl slightly decrease the decay rate of the Tb-4 complex. In the presence of electron-rich hydrocarbons, the half-life increases to 1.45-1.55 ms. By contrast, electron-deficient aromatics such as dinitrobenzene, and dinitrotoluene significantly reduce the half-life by up to 30 %, Table 2. In all cases, the transient emission data is well fit by a single exponential decay curve, indicating that only one species in solution is responsible for emission. In these experiments, the aromatic analyte is in 3-4 fold excess of the fluorescent reagent Tb-4.

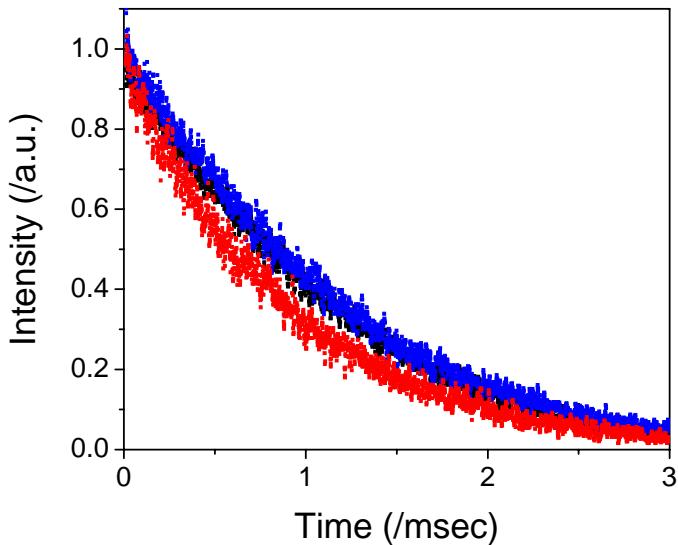


Figure 5. Normalized fluorescent decay curves of 545 nm emissions of (black) Tb-4, (blue) Tb-4 and toluene, and (red) Tb-4 and *o*-dinitrobenzene in methanol (Concentrations of Tb-4 are 0.1 mM and the toluene and dinitrobenzene are at 30 ppm)

Table 2. Half-lives for Tb-4 in the presence of electron-rich and -deficient aromatics*

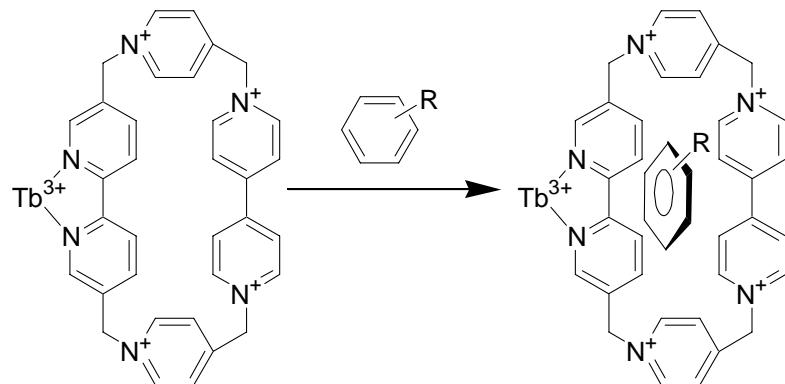
Electron-Deficient Aromatics		Electron-Rich Aromatics	
dinitrotoluene	0.90 ms	toluene	1.55 ms
<i>m</i> -dinitrobenzene	0.96 ms	naphthalene	1.46 ms
<i>o</i> -dinitrobenzene	0.92 ms	biphenyl	1.49 ms

* Tb-4 in methanol has a half-life of 1.36 ms

Importantly, these results demonstrate that the cyclophane assay Tb-4 can distinguish between the target chemicals (nitroaromatics) and interferents (hydrocarbons). Binding constants for Tb-4 and the various analytes were not obtained due to the lack of a well-defined spectroscopic signature for the supramolecular complexes. The mechanism for detecting and identifying the different analytes is depicted in Scheme 3. Presumably, the aromatics dock into the cyclophane cavity. Without the cyclophane present, the lifetime of the Tb³⁺ is independent of the analyte. Upon binding of the analyte, the photophysical properties of the Tb-4-aromatic complex are significantly altered from the parent Tb-4.

However, the specific cause of the changes can be due to a number of factors including a geometric change in the cyclophane structure, a change in the efficiency of quenching, or perhaps the analytes themselves provide new quenching mechanisms.

Scheme 3



Changes in the absorption energies of the charge-transfer band provide a possible cause for the variations in the excited state lifetimes (Figure 6). Energy overlap between the 545 nm emission and the charge-transfer absorption suggests that the charge-transfer process associated with the cyclophane is energetically suited to quench the terbium emission. Subtle changes in the spatial positioning of the Tb center and the donor and acceptor positions involved in the charge transfer can significantly alter the efficiency of the quenching mechanism. From the absorption spectra, it is clear that the intensity of the charge-transfer absorption is highly dependent on the analyte. As the analyte perturbs this process, possibly by occupying the space between the donor and acceptor rings in the cyclophane, the quenching of the Tb emission is similarly changed. However, unlike the obvious trend in the half-lives, in which electron-rich aromatics decrease the decay rate and electron-poor aromatics increase the quenching process, there are no apparent trends in the absorption energy of the charge-transfer band. Less evident trends such as changes in the spatial relationship between the terbium and the quencher due to the analyte molecule likely exist and these associations can dictate the observed lifetime trends.

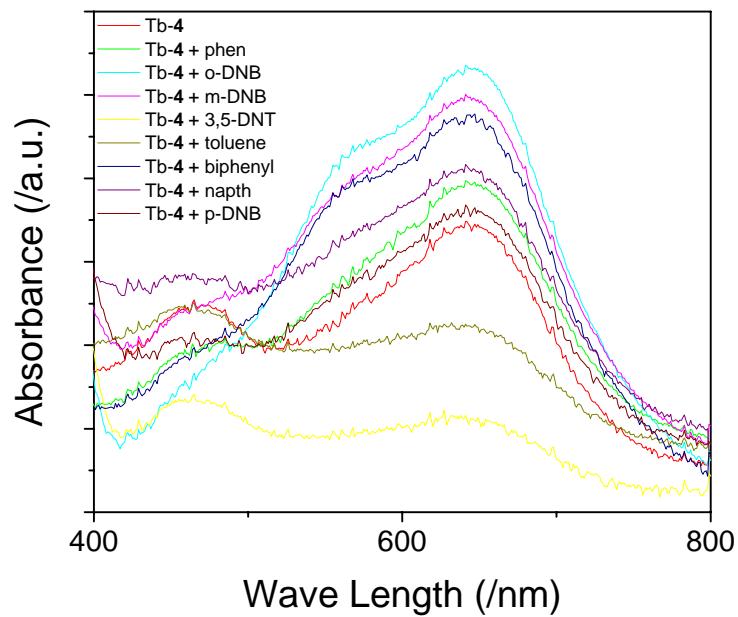


Figure 6. UV-Vis spectra of Tb-4 in methanol and in the presence of a variety of aromatics including both electron-rich and electron deficient (Concentrations of Tb-4 are approximately 0.03 mM with a 10 fold excess of the aromatic)

5. CONCLUSIONS

The fluorescent receptor Tb-4 has been successfully synthesized and demonstrated the ability to differentiate nitroaromatics that mimic explosives and propellants from innocuous aromatic hydrocarbons. While the hydrocarbons which may be present in the soil of test ranges cause an increase in the lifetime of the fluorophore, *the target analytes successfully quench the Tb-4 emission*. Preliminary test prove that concentrations of nitroaromatics as low as 9 ppm could be detected. However, the lower detection limit has not been fully probed. Due to the relatively high intensities of the emission, it is likely that the true detection limit will be several orders of magnitude better. Future work will focus on quantifying the sensitivity range of the Tb-4 reagent. *These results establish Tb-4 as a high quality chemical assay for detecting nitroaromatics.*

To proceed with this new technology, several questions must be addressed concerning the assay's applicability:

- (1) How do interferents affect the reliability of the assay?
- (2) What are the detection limits?
- (3) Is the assay applicable to perchlorate detection?
- (4) Does the Tb-4 assay respond to nitramines (HMX, RDX)?

To address these questions, we propose to continue this program for one more year. Although the assay has been successfully demonstrated to detect and identify nitroaromatics, a more thorough understanding of the ability of Tb-4 to distinguish explosives and propellants from innocuous backgrounds would be fruitful. Each issue can be adequately studied in the next year. Importantly, additional studies promise to expand the scope of the Tb-4 test to include perchlorates and other explosives. Also, a detailed quantitative study on the limits of Tb-4 will allow the assay's results to be better interpreted.

In the first year of this program, considerable effort was spent on developing the synthesis of Tb-4 and proving its analytical capabilities. Subsequent work will focus on understanding the exact limits of this new technology. Specifically, equilibrium constants for the binding of Tb-4 with various aromatics will allow for a more precise prediction of how different background chemicals can interfere with the cyclophane. A combination of UV-vis and NMR spectroscopies can quantify the affinity of the cyclophane with different analytes. Coupling this data to the fluorescence measurements will yield the overall sensitivity limits of the assay. With a more thorough study we will be better able to predict how Tb-4 can be integrated into dip-stick or spray-on applications.

Due to time constraints we were unable to investigate how Tb-4 responds to perchlorates or nitramines (HMX and RDX). Since the charge-transfer band is highly dependent on the nature of the counter anions, it is highly likely that ClO_4^- will significantly alter the quenching properties of the cyclophane. More difficult to predict, however, is the affinity

and perturbation properties of the nitramines. There is no obvious binding affinity of Tb-**4** for these chemicals as they lack both charge and aromaticity. However, under aqueous conditions, simple hydrophobic affects and entropy may be sufficient to drive the binding of HMX and RDX. Still unknown is what effect these bindings will have on the photophysical properties of Tb-**4**. With an additional year of effort, we can address these issues and construct a more complete picture of the sensing properties of new chemical assay. This subsequent effort will not need a significant amount of synthesis as a considerable quantity of **4** is readily available. Rather, our efforts will focus on quantifying the fluorescence and binding properties of Tb-**4** with various analytes.

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